

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Wu et al.

Confirmation No: 3823

Serial No.: 10/576,491

Group Art Unit: 1652

Filed: April 18, 2006

Examiner: S. Swope

For: Protease with Improved Stability in Detergents

SUPPLEMENTAL DECLARATION OF DR. JÜRGEN KNÖTZEL UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Jürgen Knötzel, do hereby state and declare as follows:

1. I am the same Jürgen Knötzel who provided the DECLARATION OF DR. JÜRGEN KNÖTZEL UNDER 37 CFR 1.132 on 21 January 2011 ("January 2011 Knötzel Declaration") in the above-identified of patent application.

2. I understand that the U.S. Examiner has asked for clarification of the concentration of LAS-detergent used in the assay described in paragraph 5 of the January 2011 Knötzel Declaration.

3. As previously stated in the January 2011 Knötzel Declaration, LAS-detergent (Detergent 1) was prepared according to Table 1 of Isono as follows:

25% LAS: 274.7mg/ml*

40% Sodium tri-phosphate: 400mg/ml

29% Sodium sulphate: 290mg/ml

5% Sodium silicate: 50mg/ml

1% Carboxymethyl cellulose (Finnfix BDA): 10mg/ml

However, these amounts of solid ingredients were undissolvable in 100mL of 0.05M Tris-HCl pH 11 buffer. For this reason, the above-listed ingredients were combined and diluted 10-fold so that all solids would dissolve. This gave a stock of 2.5% (27.47mg/ml) LAS solution.

* The January 2011 Declaration used the European convention of a "h." to represent the decimal point. For the Examiner's convenience, I now use the American convention of a "." to represent the decimal point.

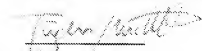
For the assay, the 2.5% (27.47mg/ml) LAS solution is further diluted according to Isono, but in order to achieve the same final dilution of LAS-detergent as Isono, we had to use 10 times more of our stock. 500 mg of 2.5% LAS solution is taken in 20 mL 0.05 TRIS-HCl pH 11. This gives a final concentration of 0.0625% (0.625mg/ml) LAS as final concentration of the assay.

4. By comparison, Isono reports preparation of a 25% LAS solution (250mg/ml). 5 mg of 25% LAS solution is taken in 2 mL of buffer to provide 0.0625% (0.625mg/ml) LAS as final concentration of the assay.

5. The concentration of LAS detergent in the assay experiments carried out under my direction and supervision is the same as the concentration of LAS detergent in the assay experiments of Isono.

6. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 13 day of October, 2011



Jürgen Knötzel